REMARKS

Claims 1, 2, 4, 5, 9, 31, 32, 40 and 42-44 are pending and are under examination (claims 33-39 and 41 having been canceled and claims 42-44 added by the present amendment; claims 3, 6-8 and 10-30 were previously canceled). Claims 1, 2, 4, 5, 9, 31, 32 and 40 have been amended. The amendments to the claims are supported by the specification as filed at, *e.g.*, page 15, line 31, to page 16, line 15. New claims 42-44 are supported by the specification as filed at, *e.g.*, page 16, lines 6-7, and page 19, lines 10-15. No new matter has been added.

Claim 1 -- formalities

Claim 1 has been amended to delete "the" in line 1, which was an inadvertent typographical error.

Objections under 35 U.S.C. § 132

The Examiner objects to the amendment to the specification filed January 30, 2004, for allegedly adding new matter. The Examiner states that "[t]he amendment for incorporated by reference to 'U.S. application [*sic.*] U.S. Patent No. 5,840,299' on page 23, line 11 of the specification does not enjoy the status of part of the original disclosure in the application" (Office Action at page 2). In an effort to advance prosecution, Applicants have amended the specification to remove the reference to U.S. Patent No. 5,840,299. Applicants respectfully request this objection be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejects claims 37 and 40-41 as indefinite. The Examiner states that these claims "stand indefinite in the recitation of 'L25' and '21.6', respectively because its [*sic.*] characteristics are not known" (Office Action at page 2). Although Applicants disagree, in an effort to advance prosecution, Applicants have canceled claims 37 and 41, and have amended claim 40 to remove reference to "21.6". Thus, this rejection should be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner rejects claims 34-41 "as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention" (Office Action at page 3). Although Applicants disagree, claims 34-39 and 41 have been canceled, and claim 40 has been amended to remove reference to "21.6". Thus, Applicants respectfully request this rejection be withdrawn.

Rejections under 35 U.S.C. § 103(a)

I. Van Zaanen combined with other references

Claims 1, 2, 4, 5, 9, 31, 32 and 34-39 stand rejected as unpatentable over Van Zaanen *et al.*, *Br. J. Haematol.* 102:783-790, 1998 ("Van Zaanen") in view of Masellis-Smith *et al.*, *Cancer Res.* 57:930-936, 1997 ("Masellis-Smith") and Lokhorst *et al.*, *Blood* 84:2269-2277, 1994 ("Lokhorst") and Owens *et al.*, *J. Immunol. Methods* 168:149-165, 1994 ("Owens"). Claims 34-39 stand rejected as unpatentable over Van Zaanen in view of Masellis-Smith and Lokhorst and Owens further in view of U.S. Patent No. 5,932,214 ("Lobb") and Kamata *et al.*, *Biochem. J.* 305:945-951, 1995 ("Kamata"). Applicants respectfully traverse the rejection.

The Examiner rejects the Declaration under 37 C.F.R. § 1.131, filed January 30, 2004, as insufficient because it did not state the invention was conceived and reduced to practice in this country, a NAFTA country, or a WTO country. Applicants provide herewith a new Declaration that rectifies this defect, as Exhibit 2.

The primary reference for this part of the rejection is Van Zaanen. Van Zaanen, as shown by the date stamped copy submitted herewith as Exhibit 1, has a publication date of August 18, 1998. Applicants reduced the claimed invention to practice in this country prior to the effective publication date of this reference, as shown by the enclosed Declaration under 37 C.F.R.§ 1.131 of all the inventors. Therefore, Van Zaanen is not available as prior art against the present

claims. Without Van Zaanen, a *prima facie* case of obviousness cannot be made. Accordingly, Applicants respectfully request that this rejection be withdrawn.

II. Lee in view of Lobb and Kamata

Claims 1, 2, 4, 5, 9, 31, 32 and 34-39 stand rejected as unpatentable over U.S. Patent No. 6,495,525 ("Lee") in view of Lobb and Kamata. Applicants respectfully disagree.

As discussed in the Amendment filed January 30, 2004, Lee discloses the use of a small molecule VLA-4 inhibitor (oMePUPA-V) to treat animal models of pulmonary inflammation (airway hypersensitivity) and delayed type hypersensitivity. Lee suggests that the small molecule inhibitor could also be used to treat "VLA-4-mediated cell adhesion and pathologies associated with that adhesion, such as inflammation and immune reactions" and lists 20 specific disorders within that class. Lobb and Kamata disclose various anti-VLA-4 antibodies but do not relate to treatment of multiple myeloma.

According to the Examiner,

> [Lee] teaches anti-VLA-4 monoclonal antibodies which have been shown to inhibit VLA-4 dependent adhesion interactions both in vitro and in vivo. Further, [Lee] administered the anti-VLA-4 antibody (PS/2) in activity in models of delayed type hypersensitivity. Further, [Lee] teaches that the anti-VLA-4 antibody PS/2 inhibited swelling by approximately 30% whereas oMePUPA-V administered enterally was without effect in this model (see col., 22 lines 28-52, under example 3 in particular). Therefore, [Lee] teaches anti-VLA-4 antibody that inhibited swelling in vivo which mimic the function of oMePUPA-V (Office Action at page 5).

The Examiner concludes that one of ordinary skill in the art would have been motivated to substitute an anti-VLA-4 antibody for oMePUPA-V. Applicants respectfully disagree.

First, Applicants note that the disclosure in Lee that anti-VLA-4 antibodies have been shown to inhibit VLA-4 dependent adhesion interactions both in vitro and in vivo appears in the background (*see* col. 1, lines 58-60). Moreover, Applicants note that Lee discloses experiments that treat animal models of pulmonary inflammation (airway hypersensitivity) and delayed type hypersensitivity (Lee, Examples 2-4). Lee lists a broad range of other immune and inflammatory

diseases that can be treated with oMePUPA-V and also lists multiple myeloma and tumor metastasis. Multiple myeloma is a type of cancer (neoplasm) that develops in a subset of white blood cells but it is not an immune or inflammatory disorder *per se*, unlike the other disorders listed in Lee or the disorders treated in the *in vivo* examples in Lee. There is no motivation to select multiple myeloma from this long list in Lee to treat with an antibody. A skilled artisan would certainly not be motivated to use an antibody therapeutic to treat a neoplasm based on Lee's data showing that a small molecule drug against a target can be used to treat a disorder related to inflammation, or more particularly, to a hypersensitivity-type inflammatory response. Treating neoplasms with antibodies is a completely different area of medicine than treating immune- or inflammatory-mediated diseases with small molecule drugs. Thus, one of ordinary skill in the art would not have been motivated to use an anti-VLA-4 antibody to treat multiple myeloma, based on the references argued by the Examiner.

Further, one of skill in the art would have no reasonable expectation of success, based on Lee in view of Lobb and Kamata, to use an anti-VLA-4 antibody to treat multiple myeloma. As discussed in the Declaration under 37 C.F.R. § 132 of Dr. Blake Pepinsky (hereinafter "Pepinsky Declaration"), submitted with the Amendment filed January 30, 2004, antibodies are completely different than small molecules.

First, antibodies as a class of agent differ greatly in terms of structure from small molecules (see Pepinsky Declaration at paragraph 4). For example, antibodies are vastly different in size than small molecule drugs such as oMePUPA-V. This structural difference (as well as others discussed below) means that antibodies work very differently. They act, as shown in the Pepinsky Declaration, by very different mechanisms, mechanisms that may well not give the same result. Thus, they have different structures and different mechanisms of action.

This is not a case where one can simply conclude that, if antibodies bind and work, and small molecules bind and work, then the two are interchangeable. As discussed in the Pepinsky Declaration, due to its small size, a small molecule works in very different ways. A small molecule drug is typically directed to a "pocket" or specific docking site on the target molecule,

where it may act as either an agonist or an antagonist. In contrast, antibodies are large molecules that, although they bind to a particular epitope, their structural attribute (size) means they work in very different ways than small molecules, and effectively cover a large surface area and thereby act to block a biological pathway though stearic hindrance, as opposed to binding a specific active site or pocket. As discussed in the Pepinsky declaration, oMePUPA-V binds at the ligand binding site and therefore may act as an agonist. In contrast, none of the existing anti-α integrin antibodies bind directly at the ligand binding site. For this reason alone, a skilled practitioner would not have believed that oMePUPA-V, which differs greatly in structure from a small molecule, would be interchangeable with an anti-α4 integrin antibody.

The structural and functional differences do not end with those discussed above. Antibodies have a structure referred to as an Fc receptor; the small molecules of the cited art do not have this structure (see Pepinsky Declaration, paragraph 5). Thus, in contrast to oMePUPA-V, an antibody-based therapeutic would be expected to implicate aspects of the immune response in its effect. As discussed in detail in the Pepinsky Declaration, the binding of Fc receptors by the Fc domain of an antibody molecule provides signals that activate and recruit immune and inflammatory cells, or, alternatively, that send inhibitory signals that downregulate immunity. The implication of additional immune mechanisms with an antibody could result in a completely different effect in vivo than that of oMePUPA-V. Even if an antibody and a small molecule were alike in all other respects, this difference in activity alone would mean they are not interchangeable. Thus, a skilled artisan would not have reasonably predicted that an anti-α4 integrin antibody, which has an Fc receptor structure, would have the same effect as oMePUPA-V, which lacks such a structure, in vivo. Such antibody-specific mechanisms are an important reason why an antibody and a small molecule would not be considered interchangeable.

The Examiner "realizes the difference between the antibodies and the small molecule drugs mechanism of action, however, the issue is the obviousness for one ordinary skill in the art at the time of the invention was made to use the VLA-4 inhibitor to treat MM" (Office Action at page 6, emphasis added). Applicants respectfully disagree with the Examiner's characterization of the issue. The issue is whether, based on the disclosure of the use of a small molecule to treat

multiple myeloma, it would have been obvious to one of skill in the art to use an anti-VLA-4 antibody to treat multiple myeloma, and not a VLA-4 inhibitor in general. Applicants have outlined, as the Examiner has realized, the differences between antibodies and small molecules. As discussed above, antibodies and small molecules are different in their structures, as well as in their mechanisms of action. Thus, as stated in the Pepinsky Declaration, a skilled artisan would not have reasonably predicted that an anti-α4 integrin antibody would have the same effect as oMePUPA-V in vivo.

The activity or function of a molecule is dictated by its structure. The antibody structure allows binding to a different spectrum of targets than does the structure of the small molecule. Moreover, there is yet another structure/function difference. Anti-α4 integrin antibodies, as recited in the claims, have a structure that imparts a different specificity than oMePUPA-V (see Pepinsky Declaration at paragraph 6). Lee teaches that oMePUPA-V is highly specific for VLA-4 (having α4/β1 subunits) but does not act on α4/β7 integrin (Lee 7:39-42; 25:33-34). In contrast, the α4 integrin antibodies recited in the claims can bind both α4/β1 and α4/β7, implicating an additional integrin pathway. The broader specificity of an anti-α4 integrin, compared to oMePUPA-V, would have made it unpredictable that an anti-α4 antibody structure would have the same effect as oMePUPA-V small molecule structure in vivo at all, much less have the same applicability across such a broad range of disorders. Even if antibodies and small molecules were exactly alike in every other way, this difference in specificity would show they are not interchangeable.

Finally, the only side-by-side comparison of antibodies and small molecules in the cited art is in Example 3 of Lee. In Example 3, Lee compared the use of an anti-VLA-4 antibody to the use of oMePUPA-V to treat animal models of delayed type hypersensitivity. In this example, the antibody was effective, but the small molecule was not. Thus, Lee specifically teaches that an anti-VLA-4 antibody and oMePUPA-V are not interchangeable to treat inflammatory-mediated diseases.

The PTO, while appearing to acknowledge both differences in structure and mechanism, concludes that they won't result in patentability:

> However, the Examiner notes that the mechanism of action does not have a bearing on the patentability of the invention if the invention was already known or obvious (Office Action at page 6).

First, the Examiner is assuming that the two mechanisms give the same result. As discussed above and in the Pepinsky Declaration, that is not the case. For example, a small molecule might act as an agonist. Second, if the PTO were in possession of an anticipatory reference, it might be correct to say that merely elucidating mechanism would not confer patentability. In cases where the method is known and one simply discovers how it works, this might be true. However, the situation here is different.

The art teaches one structure, while the claims are limited to the use of a very different structure. The mechanism helps explain why the differences in structure are relevant. Thus, the PTO is misapplying a novelty analysis. In the passage quoted, the PTO says mechanism has no bearing on patentability "if the invention was already known or obvious". Assuming "known" means anticipated, the PTO's framework might be appropriate. But as mentioned, the present rejection is one for obviousness. The PTO says mechanism is irrelevant if the invention was "obvious". That, however, is the question, and the fact that two structurally different molecules work in very different ways and can give very different results, is certainly relevant to the issue of interchangeability. This is an obviousness rejection of the use of an antibody over the use of a structurally and functionally distinct small molecule.

Thus, Applicants submit that, because of the structural and mechanistic differences between antibodies and small molecules, based on Lee in view of Lobb and Kamata, one of skill in the art would have no expectation of success in using an anti-VLA-4 antibody to treat multiple myeloma.

In conclusion, the Examiner has not made out a prima facie case of obviousness, and Applicants respectfully request this rejection be withdrawn.

Obviousness-type double patenting

Claims 1, 2, 4, 5, 9 and 34-39 are provisionally rejected as unpatentable over claims 1, 2, 4, 5 and 11 of co-pending U.S.S.N. 09/943,659. U.S.S.N. 09/943,659 is now abandoned, rendering this rejection moot.

Claims 1, 2, 4, 5, 9, 31 and 32 stand provisionally rejected as unpatentable over claims 1, 2, 4, 5, 9, 11, 12, 17, 18, 20, 21, 25, 27, 34, 35, 37 and 44 of co-pending U.S.S.N. 10/086,217. Claims 34-39 are rejected as unpatentable over claims 1, 2, 4, 5, 9, 11, 12, 17, 18, 20, 21, 25, 27, 34, 35, 37 and 44 of U.S.S.N. 10/086,217 in view of U.S. Patent No. 5,932,214 and Kamata. Once the present claims are deemed otherwise allowable, Applicants will address this rejection appropriately.

Enclosed is a Petition for Extension of Time along with the required fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 23 May 2005

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Chimaeric anti-interleukin 6 monoclonal antibodies in the treatment of advanced multiple myeloma: a phase I dose-escalating study

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Summary. Interleukin 6 plays a key role in the pathogenesis of multiple myeloma (MM). Therefore we conducted a phase I dose-escalating study with chimaeric monoclonal anti-IL6 antibodies (cMab) in MM patients resistant to second-line chemotherapy. The cMab (CLB IL6/8; K_d $6{\cdot}25\times10^{-12}$ M) was given in two cycles of 14 daily infusions, starting on day 1 and day 28, repectively, with a daily dose of 5 mg in patients 1–3, 10 mg in patients 4–6, 20 mg in patients 7–9 and 40 mg in patients 10–12 (total dose 140 mg, 280 mg, 560 mg and 1120 mg of anti-IL6, respectively). 11/12 patients had elevated pretreatment IL6 levels.

Except for transient thrombocytopenia in two patients there was no toxicity. There were no changes in haemoglobin levels, granulocyte count, liver enzymes or renal function. No human anti-chimaeric antibodies were induced. This was also reflected in a long half-life time of the cMab (median 17·8 d), resulting in accumulation of the anti-IL6 cMab and high levels of circulating IL6. However, this was in the form of biologically inactive IL6/cMab complexes and did not result in acceleration of the disease. Although C-reactive protein (CRP) levels were decreased to below detection level in 11/12 patients, indicating effective IL6 blocking, none of the patients achieved a response according to the standard criteria. We conclude that this chimaeric anti-IL6 Mab has a low toxicity, low immunogenicity and a long $T_{1/2}$. A dose of 40 mg/d for 14 d can safely be used in future phase II studies.

Keywords: chimaeric, anti-interleukin 6, multiple myeloma.

Interleukin-6 (IL6) is a cytokine with multiple biological activities. It has been shown to be involved in such diverse processes as T-cell activation, induction of acute-phase proteins, and stimulation of haemopoietic precursor cell growth and differentiation (Heinrich *et al*, 1990; Kishimoto, 1989). In the last decade *in vitro* and *in vivo* observations have suggested a major role of IL6 in the pathogenesis of multiple myeloma (MM) (Anderson *et al*, 1989; Hilbert *et al*, 1995; Kawano *et al*, 1988; Klein *et al*, 1989; Lokhorst *et al*, 1994; Nordan *et al*, 1987; van Oers *et al*, 1993; Zhang *et al*, 1989). Especially in patients with active and/or terminal disease, serum IL6 levels have been found to be elevated (Bataille *et al*, 1989; Klein *et al*, 1990).

Murine anti-IL6 Mab have been used (in rather heterogenous schedules) in the treatment of myeloma patients (Bataille *et al*, 1995; Klein *et al*, 1991). No major side-effects have been observed, but antibodies to mouse immunoglobulin (HAMA) were frequently detected about 2 weeks after starting the treatment. This resulted in rapid Mab clearance and diminished efficacy of this treatment. These HAMAs are frequently directed against the Fc part of the mouse immunoglobulin (Hoffman, 1990), and may also induce anaphylactic reactions.

In order to reduce the risk of induction of HAMAs and thus to increase efficacy of the treatment, we produced a chimaeric anti-IL6 antibody (cMab). With this cMab we performed a phase I dose-escalating study in MM patients who were resistant to second-line chemotherapy. Here we report the results of this phase I study.

PATIENTS AND METHODS

All patients had MM according to the criteria of Durie & Salmon (1975), and had relapsed after, or were resistant to, second-line chemotherapy (VAD (vincristine, adriamycin

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Table I. Patients' characteristics.

Pt	M/F	Age (yr)	M-protein	Stage	CRP	IL6	β2M
1	M	53	IgGκ	IIIa	8	50	1·9
2	F	70	λ	IIIa	<3	3	4·2
3	F	58	IgGκ	IIIa	3	7	4·4
4	F	71	IgGκ	IIIa	12	22	7·7
5	F	74	IgAκ	IIIa	6	17	5·4
6	F	63	IgGκ	IIIa	4	13	1·5
7	F	60	IgAλ	IIIa	6	10	3·3
8	F	58	IgGκ	IIa	<3	41	2·4
9	F	54	IgGκ	IIIa	4	7	4·0
10	F	53	IgGλ	IIIa	<3	5	2·9
11	M	69	IgAκ	IIIa	6	33	2·8
12	F	64	*	IIIa	76	9	10·2
Median		61·5			5	11·5	3·7

CRP, C-reactive protein (normal value <5 mg/l); IL6, interleukin-6 (normal value <3 pg/ml); β2M, beta-2 microglobulin (normal value 1·1–2·4 mg/l).

* Non-secretor.

and dexamethasone) or VAD-like regimens, intermediate to high doses of melphalan; 70–140 mg/m^2 with or without autologous bone marrow or peripheral stem cell support).

Exclusion criteria were; age <18 or >75 years, life expectancy <3 months, Karnofsky score <60, diabetes mellitus, hypercalcaemia requiring treatment, recent allogeneic bone marrow transplantation, creatinine level >150 μmol/l, co-existing malignancies and active infection.

Pretreatment characteristics of these 12 patients are shown in Table I.

Chimaeric monoclonal anti-IL6 antibody. A murine-human chimaeric anti-IL6 monoclonal antibody (chimaeric CLB IL6/8) was developed, containing the antigen-binding variable region of the murine anti-IL6 antibody (CLB IL6/8) (Brakenhoff *et al*, 1990) and the constant region of a human IgG1 kappa immunoglobulin. The neutralizing Mab CLB IL6/8 blocks binding of IL6 to the IL6 receptor (CD 126) (Ehlers *et al*, 1994) and has a high affinity for recombinant as well as native IL6 ($K_d = 6{\cdot}25 \times 10^{-12}$ M) (Brakenhoff *et al*, 1990; van Zaanen *et al*, 1996). The chimaeric Mab was manufactured by Centocor, Leiden, The Netherlands.

Treatment schedule. After obtaining written informed consent according to the guidelines of the participating institutes, each patient received two cycles of treatment with cMab. Both cycles (starting at day 1 and 28 respectively) consisted of 14 daily 2 h i.v. infusions of the cMab (see Fig 1). This schedule was chosen to study the possible occurrence and response to re-treatment of a rebound phenomenon (i.e. acceleration of disease activity following cessation of the antibody administration) as has been described in patients treated with murine anti-IL6 Mab at the moment therapy was stopped (Klein *et al*, 1991). Before each cycle a test dose (10 μg) was given by slow i.v. push over 5 min. As none of the 12 patients developed an immediate hypersensitivity reaction, in all cases treatment was started 15 min later. The first three patients received a daily dose of 5 mg of the cMab (total dose 140 mg), the next three patients received 10 mg/d (total dose 280 mg), patients 7–9 received 20 mg/d (total dose 560 mg) and the last three patients received 40 mg/d (total dose 1120 mg).

Levels of IL6 and anti-IL6 antibodies. During treatment with the chimaeric CLB IL6/8 almost all the IL6 in the plasma circulated as a complex with the antibody (van Zaanen *et al*, 1996). IL6 levels were determined with the B9 bioassay as described before (Aarden *et al*, 1987; van Zaanen *et al*, 1996). One unit of B9-stimulating activity was defined as the amount inducing half-maximal proliferation and corresponded to 1 pg of IL6. To determine the total IL6 level (i.e. free IL6 plus IL6 complexed to cMab), an excess (10 μg/ml) of CLB IL6/14 was added to each well in order to displace IL6 from its binding to the *in vivo* administered neutralizing chimaeric CLB IL6/8. CLB IL6/14 and CLB IL6/8 Mab recognize partly overlapping sites of IL6. However, CLB IL6/14 is not capable of inhibiting IL6 activity in the B9 bioassay (Aarden, 1991). During treatment with the cMab actual free IL6 levels cannot be measured, because the dilution of the samples necessary for testing in the B9 bioassay or ELISA immediately influences the equilibrium between IL6–cMab complex, free IL6 and free cMab. Therefore free IL6 levels were calculated using the Henderson-Hasselbalch equation, with the K_d, the daily serum cMab levels and the total IL6 levels as known parameters.

Levels of the chimaeric CLB IL6/8 monoclonal antibody were determined using a radioimmunoassay as described in detail before (van Zaanen *et al*, 1996). The threshold of this assay is 0·5 ng/ml of antibody.

Human anti-chimaeric antibody (HACA) levels were determined using an ELISA. Briefly, the cMab (CLB IL6/8, IgG1-kappa) was coated overnight at room temperature (2 μg/ml in 100 μl well) on flat-bottomed microtitre plates. The plates were washed four times with PBS/Tween solution. Patients

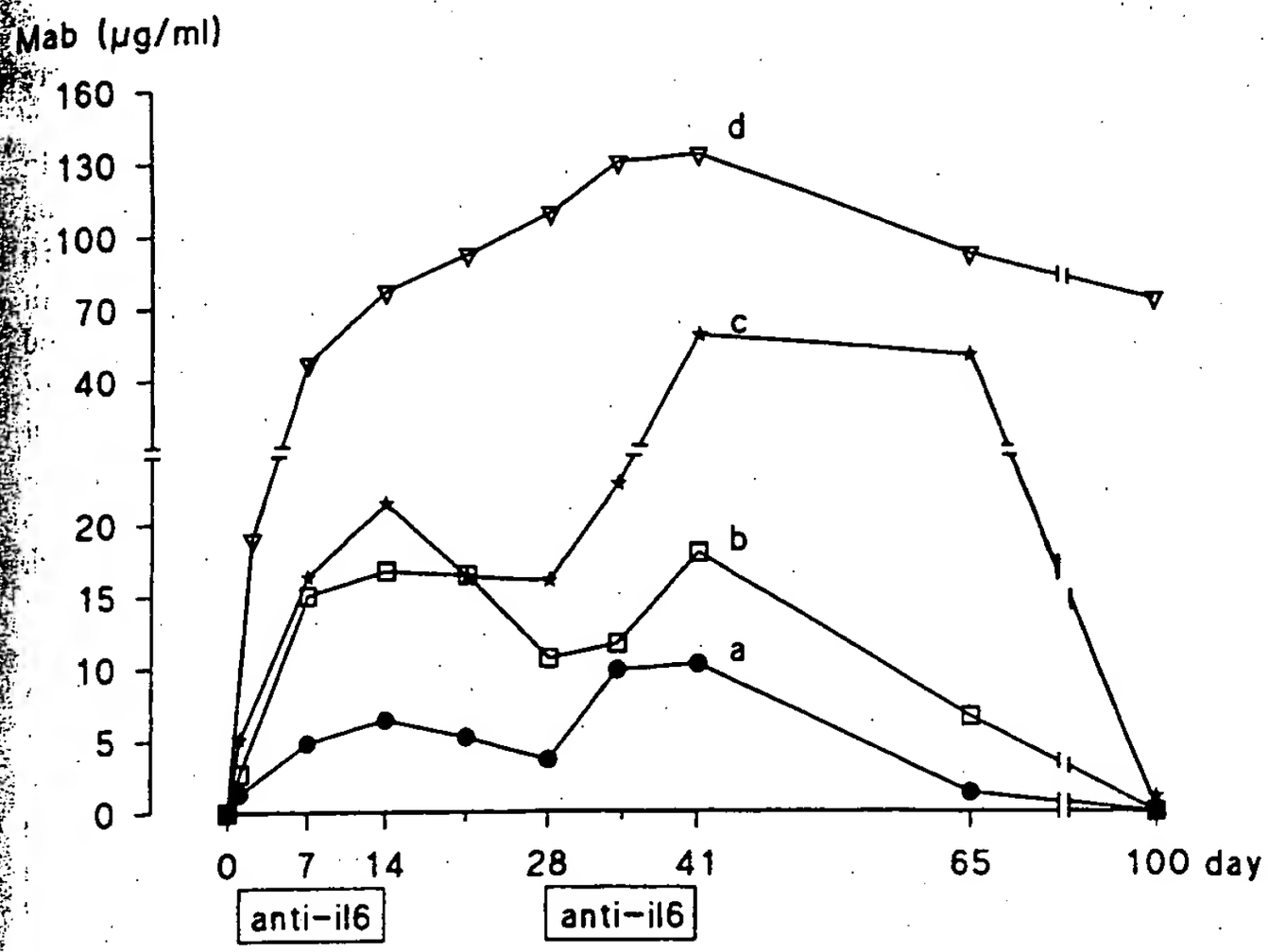

Fig 1. Mean serum levels of chimaeric anti-IL6 Mab in the different dosage groups. Patients 1–3 (a) = 5 mg/d: patients 4–6 (b) = 10 mg/d; patients 7–9 (c) = 20 mg/d: patients 10–12 (d) = 40 mg/d. Patient 12 received treatment on days 1–4 and 16–29. Day 65: $n = 9$. day 100: $n = 5$.

sera were added in different dilutions (1/50 up to 1/800) in HPE-buffer (CLB. Biotechnology. Amsterdam. The Netherlands) and incubated for 2 h at room temperature. After washing. the plates were incubated with HRP-conjugated monoclonal mouse anti-human lambda light chain (KH29. CLB. Amsterdam. The Netherlands) in 100 µl HPE buffer for 1 h at room temperature. Subsequently. after washing. the bound peroxidase was detected at 450 nm in a Titertek Multiskan.

C-reactive protein (CRP) was determined by nephelometry (Behring. Germany): detection level 3 mg/l; normal value <5 mg/l.

Beta-2 microglobulin (β2M) was determined by a microparticle enzyme immunoassay (Abbott Laboratories, U.S.A.): normal value 1·1–2·4 mg/l.

Paraprotein (M-protein) levels were determined once weekly by. immunonephalometry (Behring Nephalometer 100 Analyzer; Behring Diagnostics, Amsterdam. The Netherlands).

Levels of IL6 and the cMab were determined daily from day 0 until day 14 and day 28 until day 41 (samples were drawn before starting infusion of anti-IL6). and on days 17. 21. 44. 48. 56 and 100.

Response to treatment was defined as a decrease in M-protein level of >50% or a decrease in plasmacytoma size in patient 12.

RESULTS

Dosage of chimaeric anti-IL6

Twelve patients (three in each dosage group) completed at least one treatment cycle of 14 d. Eight of them also completed a second 14 d treatment cycle. In the other four patients anti-IL6 treatment was stopped because they fulfilled a predefined stopping criterion: patient 1 required radiotherapy for neurological complications at day 30. Patient 5 had fever and an urinary tract infection when admitted for the second treatment cycle on day 28. In patient 9 anti-IL6 was stopped at day 34 because of pneumonia and septicaemia. In patient 12 (receiving anti-IL6 until day 5) treatment was interrupted because of fever. With antibiotic treatment her clinical condition improved and anti-IL6 was given from day 16 to day 29 (one treatment cycle of 14 d). A second cycle was not given at the patient's request.

Pretreatment values of IL6. CRP and B2M are shown in Table I. In all but one patient pretreatment IL6 levels were elevated. 6/12 patients had elevated CRP levels. There was no correlation between CRP and IL6 levels ($r = -0{\cdot}13$) in these patients. Serum β2M was increased in nine patients. Patient 12 had a non-secreting myeloma, with large plasmacytomas on the chest wall and the right upper arm. The plasmacytoma on the chest wall was measured bidimensionally, before and daily during anti-IL6 treatment. Before treatment it had a diameter of 14 × 16 cm and a growth rate of 1·5 cm/week in two perpendicular directions.

Levels of cMab and IL6

Data regarding the pharmacokinetics have been published previously (van Zaanen *et al.* 1996). The median half-life time of this cMab was 17·8 d (range 7·8–39·7). with a median distribution volume of 6·0 litres (range 3·0–9·7). Peak serum levels of the cMab ranged between 6·7 µg/ml (patient 1) and 288 µg/ml (patient 11). No HACAs were found in any of the patients during the study period of 100 d. During treatment. accumulation of the cMab occurred due to its long half-life time (Fig 1). This resulted in high total serum IL6 levels. complexed with the cMab (van Zaanen *et al.* 1996). Calculated free IL6 levels decreased to <0·5 pg/ml during treatment in all patients (Fig 2).

Toxicity

During and after administration of the cMab. no changes in

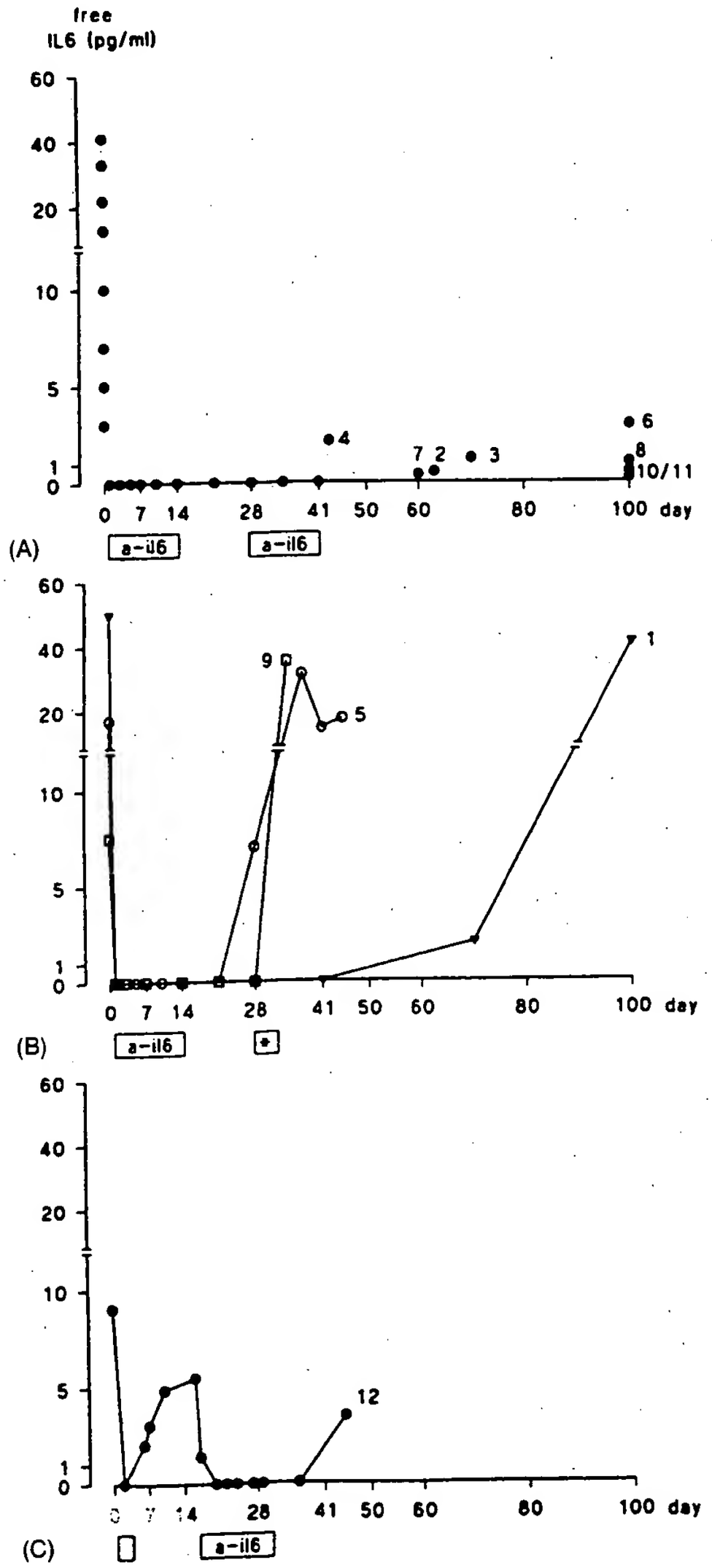

Fig 2. Free IL6 levels. (A) The eight patients who received two complete treatment cycles. Free IL6 levels <0·3 pg/ml during treatment in all patients. (B) The three patients who completed one treatment cycle of 14 d (see Results). Patient 1: anti-IL6 until day 30; patient 5: anti-IL6 until day 14; patient 9: anti-IL6 until day 34. (C) Patient 12 received anti-IL6 on days 1–4 and 16–29. Because of fever of unknown origin, anti-IL6 treatment was interrupted at day 5.

blood pressure, pulse rate or temperature were observed. These parameters were measured every 15 min for 2 h immediately after the administration of anti-IL6. No changes in liver function were found. Creatinine levels fluctuated in patients 2, 5, 10 and 12 but remained <150 μmol/l; in the other eight patients no changes in renal function were found. Haemoglobin levels remained stable, while minor changes occurred in platelet and leucocyte counts. In patients 2 and 5 we observed a transient thrombocytopenia with a nadir of 24 and 58×10^9/l respectively (on days 56 and 14, respectively). Before anti-IL6 treatment both patients had hypocellular bone marrow aspirate and biopsy (with a decreased number of megakaryocytes). In 6/12 patients (patients 2, 4, 7, 8, 9 and 11) a mild transient granulocytopenia was documented after one dose of anti-IL6. The median decrease in the number of granulocytes was 32·5% (range 20–46%), with a median time to nadir of 1 d. The granulocytes returned to base-line levels within 2 d. No alterations in lymphocyte subsets were found.

Three patients developed infectious complications; patient 5 (with known recurrent urinary tract infections) had fever with low blood pressure due to a urinary tract infection with *Escherichia coli* on the day she was admitted for the second treatment cycle. Patient 9 died because of pneumonia and septicaemia with *Staphylococcus aureus*. Patient 12 developed fever of unknown origin on day 5. She was treated with antibiotics; all cultures remained negative.

Clinical effects

In all but one patient the M-protein level did not change during both cycles of anti-IL6 treatment. In eight patients (patients 1, 3, 4, 7–11) this indicated an unaffected disease course because they had similar M-protein levels at 2 months before anti-IL6 treatment (Figs 3A and 3B). In patient 2 disease activity remained progressive during the first treatment cycle; this was reflected by a rise of M-protein and β2M levels (77% and 145% increase in relation to day 0, respectively). However, during the second treatment cycle, M-protein and β2M levels remained stable (Fig 3B). After day 60 she received 70 mg/m^2 melphalan intravenously, resulting in a temporary decrease of M-protein level. In two patients (patients 5 and 6) a marked stabilization of the M-protein occurred during therapy (Fig 3B). This was also true for the growth rate of the plasmacytoma of patient 12. In two patients (patients 1 and 7) we observed a possible acceleration in the increase of the M-protein levels after stopping anti-IL6 (Fig 3B). Likewise, after the anti-IL6 treatment was stopped in patient 12, the growth rate of the plasmacytoma was increased when compared to the growth rate before therapy (1·5 cm/week versus 2 cm/week).

Immediately after starting the anti-IL6 treatment the CRP levels decreased to below detection level in all patients except for patient 12 in which CRP decreased from 76 to 10 mg/l. Other acute-phase proteins tested, pre-albumin, alpha-1 antitrypsin and alpha-1 acid glycoprotein, remained stable and within normal ranges.

Survival

Six patients were still alive 9 months after treatment with anti-IL6. Three patients died within the study period of 100 d (patients 5, 9 and 12). Patient 5 went off study because of an urinary tract infection on day 28. After appropriate

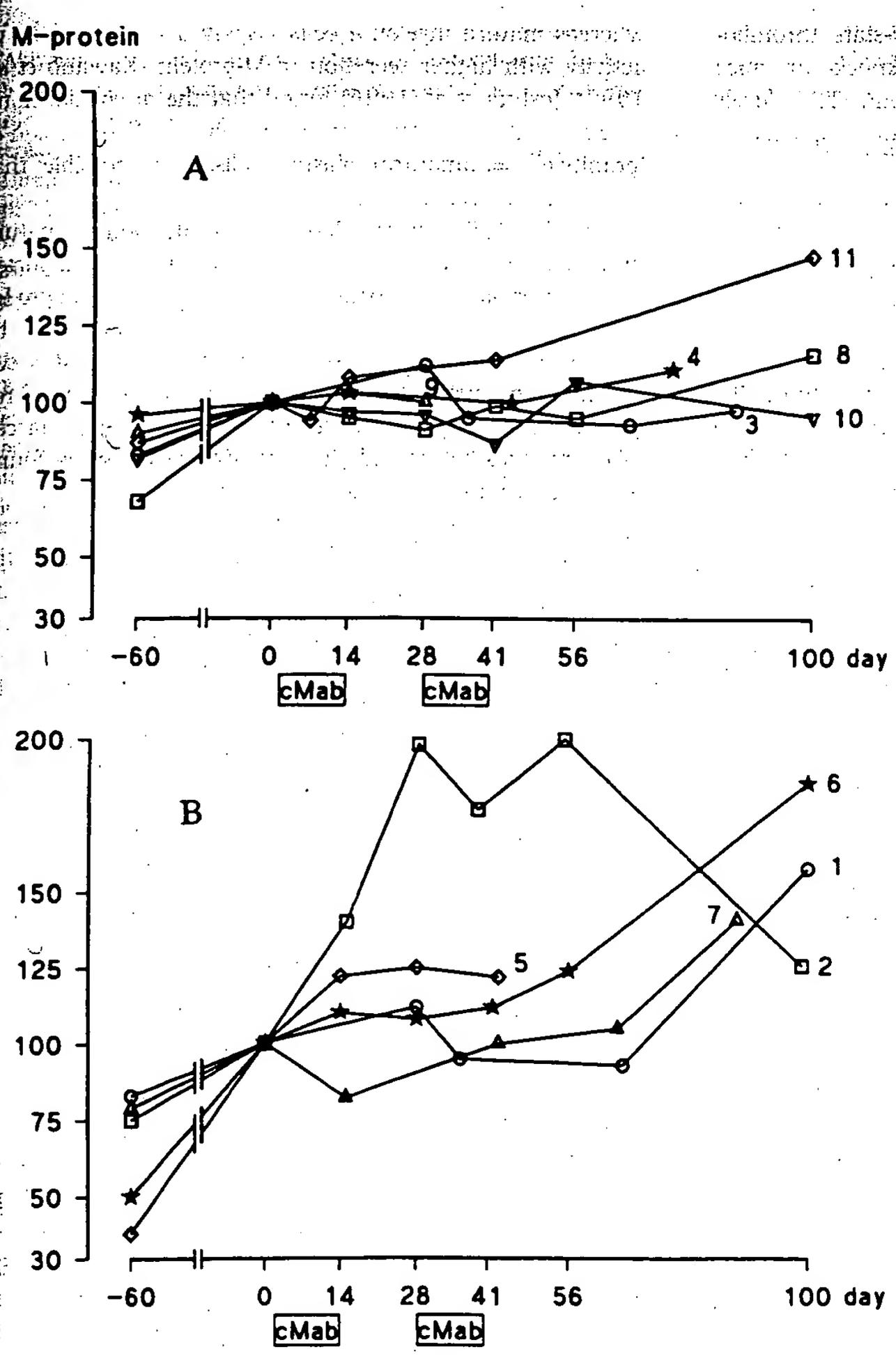

Fig 3. Percentage of M-protein levels before, during and after anti-IL6 treatment in relation to day 0 (day 0 = 100%). Anti-IL6 cMab was given in two cycles of 14 d (two boxes below abscissa). (A) Patients 3, 4, 8, 9, 10 and 11; (B) patients 1, 2, 5, 6 and 7. At day 60, patient 2 received melphalan i.v. (70 mg/m^2).

treatment she was discharged and died several weeks later at home from progressive disease. Patient 9 died on day 35 due to an irreversible septic shock. Although radiotherapy was a treatment option for the large plasmacytomas in patient 12, no further treatment was given according to the wish of the patient. She died shortly afterwards because of progressive disease with pleural effusion. Three patients died after day 100 because of refractory multiple myeloma.

DISCUSSION

From this phase I dose-escalating study with chimaeric anti-IL6 Mab in patients with advanced MM, two important conclusions can be drawn. First, the use of this cMab is safe. Despite high levels of circulating cMab, no toxicity was observed and no HACAs were induced. Second, accumulation of the high-affinity cMab resulted in high levels of circulating IL6. However, this was in the form of biologically inactive complexes and did not result in acceleration of the disease during anti-IL6 treatment.

Toxicity

Although IL6 is a multi-functional cytokine, blocking its biological activity by chimaeric anti-IL6 Mabs did not result in serious side-effects. Only a transient thrombocytopenia occurred in two patients who had hypocellular bone marrow before starting anti-IL6 treatment. The mechanism of this thrombocytopenia during anti-IL6 treatment is not completely understood. Although from studies in primates and humans it has become evident that IL6 is able to induce megakaryocyte maturation and thrombocytopoiesis (Asano *et al*, 1990; Stahl *et al*, 1991; Wickenhauser *et al*, 1995;

Stouthard *et al*, 1996), its role in steady-state thrombocytopoiesis is not clear because IL6 knock-out mice have normal platelet counts. Thrombopoietin (TPO) levels did not change during anti-IL6 treatment (unpublished observations).

Three patients developed infectious complications during therapy. Although a relationship with anti-IL6 cannot be totally excluded, it is not likely because infectious complications are common in end-stage multiple myeloma and are a major cause of death (Foerster, 1993). Moreover, in IL6 knock-out mice the outcome of TNF-induced shock was not different from IL6 wild-type mice (Libert *et al*, 1994). The three patients involved (patients 5, 9 and 12) were still able to respond to the infection with an increase of their endogenous IL6 production, resulting in increased free IL6 serum levels, fever and increased CRP levels.

High levels of circulating IL6

In agreement with other studies (Heremans *et al*, 1992; Martens *et al*, 1993; May *et al*, 1993, 1994; Mihara *et al*, 1991), we found that the use of anti-IL6 Mab led to accumulation of circulating IL6 in the form of immune complexes. An important question is whether this complexed IL6 has biological activity. If so, treatment with anti-IL6 might result in disease progression and/or clinical deterioration. Several observations make this possibility unlikely. First, during anti-IL6 treatment the CRP level (which is completely IL6 dependent; Heinrich *et al*, 1990) decreased to below detection level in 11/12 patients. In the remaining patient CRP decreased by 87% compared to the pretreatment value. Second, we did not observe an accelerated disease course during anti-IL6 treatment. However, after anti-IL6 was stopped, in three patients a possible rebound phenomenon was seen, resulting in increased M-protein levels in two patients and an accelerated plasmacytoma growth in one patient. A possible explanation might be that when free cMab levels decrease some time after stopping administration of cMab, IL6 gradually dissociates from the cMab/IL6 complex. The free IL6 level will increase according to the Henderson-Hasselbalch equation: $K_d = [IL6_{free}]\ [cMab_{free}] / [complex]$.

It has been suggested that the rebound phenomenon might be prevented by the formation of multivalent IL6/Mab complexes which are rapidly cleared from the circulation. Indeed in mice it has been found that simultaneous treatment with three anti-IL6 Mab (binding to three distinct epitopes of IL6) induced a rapid uptake of these complexes by the liver, leading to enhanced elimination of IL6 from the circulation (Montero-Julian *et al*, 1995).

Efficacy

Although this phase I dose-escalating study does not allow formal conclusions as to efficacy, it was rather striking to see that during anti-IL6 treatment we observed only minor effects on M-protein levels, whereas a profound decrease of CRP levels was found in all patients. A possible explanation might be that myeloma cells in the bone marrow of MM patients consist of both immature and mature cells. Immature myeloma cells are considered to be proliferating, whereas mature myeloma cells display a low proliferative activity with higher secretion of M-protein (Kawano *et al*, 1993). Joshua *et al* (1996) found that the *in vivo* labelling index was almost entirely determined by a subpopulation of 'primitive' (=immature) plasma cells. It is possible that myeloma cell proliferation and CRP production are blocked by anti-IL6 cMab, whereas M-protein production by mature myeloma cells is less affected. This assumption is supported by the observation by Sonneveld *et al* (1991) that *in vitro* Ig-synthesis and proliferation of MM cells are stimulated by different cytokines and that anti-IL6 can block plasma cell proliferation, but not Ig-synthesis. Indeed, for the three patients in whom we were able to analyse the plasma cell labelling index before and after anti-IL6 treatment we found a strong decrease in LI (data not shown).

The existence of IL6-independent myeloma cells in these end-stage patients might be another explanation for the minor clinical responses during anti-IL6 treatment. Decreased IL6 dependence of myeloma cells in the course of the disease has been described (Asaoku *et al*, 1988). On the other hand, Zhang *et al* (1992) have shown that in the terminal phase of MM and plasma cell leukaemia, *in vitro* tumour growth was totally dependent on IL6. Moreover, from the pleural effusion of patient 12 we were able to obtain an IL6-producing myeloma cell line. IL6 production and growth of this cell line can totally be blocked by anti-IL6 cMab (unpublished observation). Likewise, in the two patients (patients 2 and 3) in whom we were able to test it, the myeloma cells were still IL6-dependent *in vitro*.

In vitro studies showed that IL6 (which is overproduced in MM) reversed the growth arrest of dexamethasone on all myeloma cell lines tested (Juge-Morinea *et al*, 1995). Thus, neutralization of IL6 by anti-IL6 Mab might improve the response of MM patients to dexamethasone. Therefore in future studies it would be relevant to combine this chimaeric anti-IL6 Mab with, for example, dexamethasone or VAD. An additional theoretical advantage of the use of anti-IL6 antibodies could be the beneficial effects on myeloma-associated bone disease. Several data suggest that IL6, probably in synergy with IL-1 beta and TNF, also plays an important role in the pathogenesis of this aspect of the disease (Kurihara *et al*, 1990; Bataille *et al*, 1992; Roodman, 1995; Barille *et al*, 1995).

In conclusion, this phase I dose-escalating study indicated that treatment of end-stage MM patients with chimaeric anti-IL6 Mabs had a very low toxicity. A dose of 40 mg/d for 14 d can safely be used in future phase II studies.

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Attorney's Docket No.: 10274-034001 / A061

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mundy *et al.*
Serial No. : 09/805,840
Filed : March 13, 2001
Title : METHODS OF TREATING MULTIPLE MYELOMA AND MYELOMA-INDUCED BONE RESORPTION USING INTEGRIN ANTAGONISTS

Art Unit : 1644
Examiner : Maher M. Haddad, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GREGORY MUNDY AND TOSHIYUKI YONEDA UNDER 37 C.F.R. §1.131

We, Gregory Mundy, a citizen of U.S.A. residing at 3719 Morgan's Creek, San Antonio, Texas, and Toshiyuki Yoneda, a citizen of Japan residing at 3530 Hunters Sound, San Antonio, Texas, hereby declare as follows:

1. We are the co-inventors of the subject matter disclosed and claimed in the above-referenced U.S. patent application.

2. We are familiar with the present claims of the application, which are directed to methods of treating multiple myeloma. The methods include administering to an individual a therapeutically effective amount of a composition comprising an anti-alpha4 integrin antibody homolog or antigen binding fragment thereof.

3. The present application claims priority to U.S. Provisional Application 60/100,182 filed September 14, 1998.

4. Van Zaanen *et al.* (1998; *Br. J. Haematol.*102:783-90) was published August 18, 1998, as shown by the date stamped copy of the reference enclosed herewith. Van Zaanen *et al.* disclose chimeric anti-IL-6 monoclonal antibodies in the treatment of multiple myeloma.

5. Prior to August 18, 1998, we conceived and reduced to practice, in this country, the claimed invention.

6. We submit herewith, as Exhibit A, a copy of a document presented at a laboratory meeting that took place at the laboratory of Toshiyuki Yoneda, at University of Texas Health Science Center, Department of Medicine, 7703 Floyd Curl Drive, San Antonio, Texas, prior to the publication date of Van Zaanen *et al.* As discussed in detail below, Exhibit A shows evidence of actual reduction to practice of the claimed invention prior to

the effective publication date of Van Zaanen *et al.* The specific date of creation of Exhibit A has been redacted.

7. Exhibit A describes and summarizes the results of *in vivo* data showing that in mice injected with 5TGM1 multiple myeloma cells, treatment with anti-VLA-4 antibody decreased the levels of IgG2b (the antibody isotype produced by 5TGM1 myeloma cells) and the incidence of paraplegia, a symptom of multiple myeloma. Exhibit A thus shows that the idea of treating multiple myeloma in a subject with an anti-VLA-4 antibody was conceived and actually reduced to practice, in this country, before the publication date of Van Zaanen *et al.*

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Feb 25, 05
Date

Gregory Mundy

Date

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Attorney's Docket No.: 10274-034001 / A061

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mundy *et al.* Art Unit : 1644
Serial No. : 09/805,840 Examiner : Maher M. Haddad, Ph.D.
Filed : March 13, 2001
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_____
Date

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3/16/05
Date

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Exhibit A

Background

Almost all myeloma patients demonstrate α4 integrin expression.

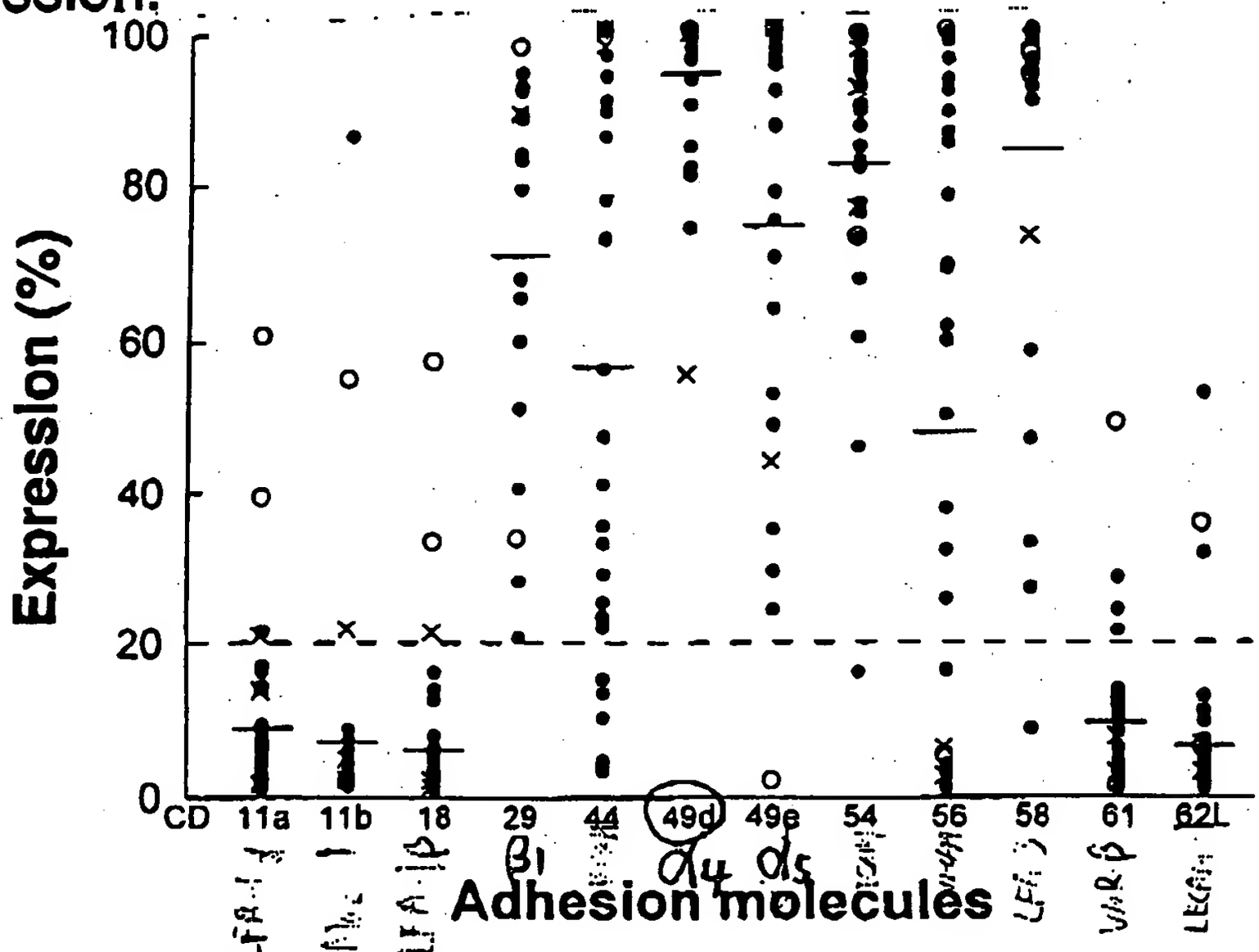


Fig. 2. Expression of adhesion molecules on freshly isolated CD38⁻ myeloma cells. The fresh myeloma cells were obtained from 26 patients with multiple myeloma (●), 2 with aggressive myeloma (○), and 3 with PCL (×). Horizontal lines represent the mean percentage expression of adhesion molecules.

(Tatsumi et al: Jpn J Cancer Res 1996)

Anti-α4β1 Ab blocks the attachment of 5TGM1 cells to ST2 and osteoclastogenesis in vitro. (Michigami)

Approach

Treat 5TGM1-bearing mice with anti-α4 integrin (PS/2)

Protocol

Exp.1 C57BL/KaLwRij mice

I PBS

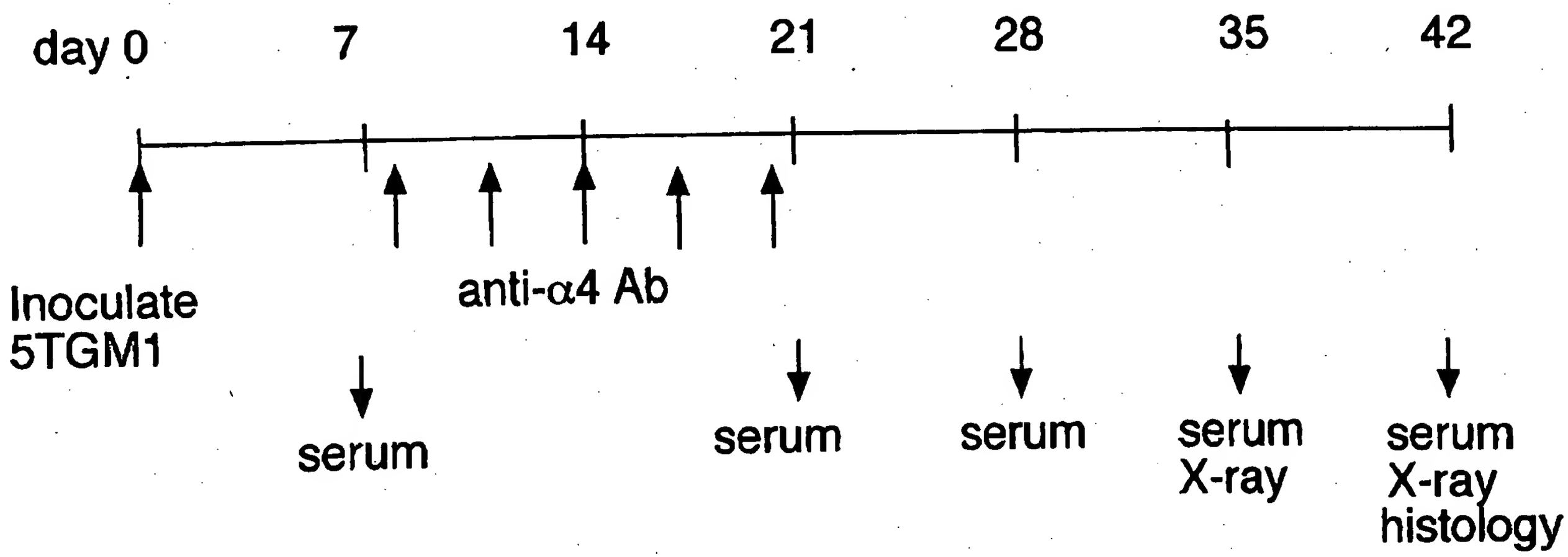
II anti-α4 40 μg

III anti-α4 80 μg

Exp.2 Nu/Bg/XID mice

I PBS

II anti-α4 80 μg



Result at 5 weeks

Exp.1 C57BL/KaLwRij mice

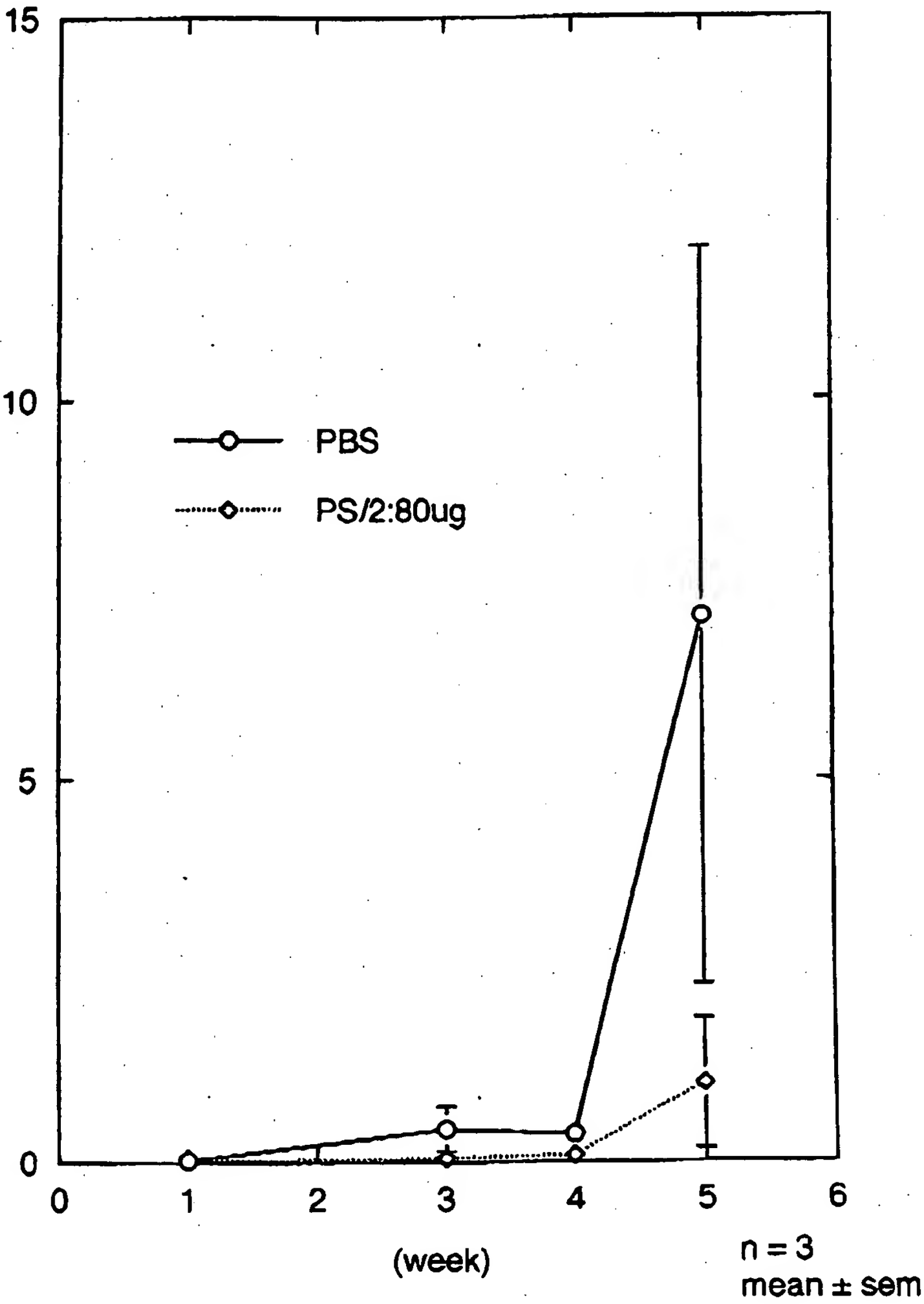
No hypercalcemia and paraplegia.

Ab-treated mice shows smaller increase of serum IgG2b than untreated mice.

Exp.2 Nu/Bg/XID mice

group	PBS			anti-α4		
mouse	a	b	c	a	b	c
paraplegia	+	-	-	-	-	-
Ca2+ (mM)	1.14	1.26	1.29	1.21	1.25	1.28
IgG2b (mg/ml)	0.40	16.54	4.51	0.27	0.06	2.70

Anti-α4 Ab Blocks IgG2b Elevation of 5TGM1-Bearing Nu/Bg/XID Mice



n = 3
mean ± sem

Conclusion

Blocking α4 integrin is a potential therapeutic approach.

Questions

Mechanism by which anti-α4 decreases IgG2b

Effects on osteolytic lesions

→X-ray, histology